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IMMUNOLOGY

AN INTRODUCTION TO MOLECULAR AND CELLULAR PRINCIPLES OF THE IMMUNE RESPONSES

SECOND EDITION

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COVER ILLUSTRATION: *Mobility of antigen-stimulated lymphocytes illustrated by a scanning electron micrograph showing a lymphocyte crawling beneath and along the edge of a fibroblast. From T-W Chang, E. Celis, H. N. Eisen, and F. Solomon, Proc. Natl. Acad. Sci. USA 76:2917, 1979.*

lished in mice, the declining level of Ag reaches an immunogenic range, causing a short burst of Ab (anti-BSA) synthesis.

2) **Abrogation by a Crossreacting Ag.** After tolerance to a T-dependent Ag, X, has been established by means of deleted or suppressed T_h cells a crossreacting Ag, X′, can terminate the tolerance. The effect is due to other T_h cells, specific for determinants that are lacking on X but present on X′.

As noted earlier, T_h cells for some determinants on a protein can help Ab-forming B cells for other determinants on the same protein (Fig. 19-14). Hence T_h cells for determinants that are unique to X′ interact with B cells for determinants that are shared by X and X′.

Thus mice that are unable to make anti-Dnp Abs in response to Dnp-ovalbumin, because they were rendered tolerant to this Ag, should make anti-Dnp Abs in response to Dnp-BγG. In another example, closer to human autoimmune disease, animals with natural tolerance to their own thyroglobulin (Tg) have been induced by immunization with denatured Tg to make Abs that react with native Tg: denaturation probably exposes new carrier determinants (which can engage new T_h cells), while preserving many determinants of native Tg (Fig. 19-14). Immunity to self-Ags (autoimmunity) is further discussed in Chapter 22.

FACTORS INFLUENCING ANTIBODY PRODUCTION IN THE WHOLE ANIMAL

The amounts and types of Abs formed vary widely with the conditions of immunization, some of which are reviewed in this section.

ROUTE OF ADMINISTRATION OF ANTIGEN

Natural Immunization. Lymphatic tissues are probably bombarded almost constantly with Ags from transiently invasive or indigenous microbes (normal flora of skin, intestines, etc.), and by those that enter the body by inhalation (e.g., plant pollens), by ingestion (e.g., foods, drugs), and by penetration of the skin (e.g., catechols of poison ivy plants). The resulting stimulation is probably responsible for the familiar histologic appearance of lymph nodes and spleen, for the normal concentration of Igs in serum (about 15 mg/ml), and for **natural Abs**—those Igs that react or crossreact (one cannot be sure which) with Ags that have not been known to serve as immunogens in the individual under test. Animals reared under **germ-free conditions** synthesize Igs at about 1/500 the normal rate, have **exceedingly low serum Ig levels** (especially of IgG), and have small, poorly developed lymph nodes and spleen.

Deliberate Immunization. For this purpose immunogens are usually injected into skin (intradermally or subcutaneously) or muscle, depending upon the volume injected and the irritancy of the immunogen. Intraperitoneal and intravenous injections are also used in experimental work, especially with particulate Ags. Regardless of the route, most Ags eventually become distributed widely throughout the body via lymphatic and vascular channels.

Because most Ags are degraded in the intestines, **feeding** is effective only under special circumstances; e.g., with attenuated poliomyelitis vaccine (Ch. 57), which can invade the intestinal wall. Allergic responses to food are probably due to Ags that resist degradation by intestinal **enzymes. Inhalation** can also be used: e.g., aerosol administration of attenuated strains of *Pasteurella tularensis*. **Preferential synthesis of IgA Abs** occurs when immunogens are introduced into the respiratory or intestinal tract, many of whose B cells are committed to produce Igs of this class.

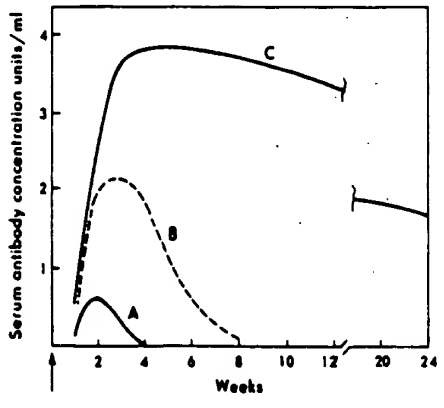
ADJUVANTS

The immunogenicity of soluble proteins is enhanced if they persist in tissues; for example, repeated small injections of diphtheria toxoid evoke a greater Ab response than the same total amount of toxoid given as a single injection. Accordingly, a widely used procedure involves the administration of inorganic gels (e.g., alum, aluminum hydroxide, or aluminum phosphate) with adsorbed immunogens so that they are released slowly for a prolonged period. The term **adjuvant** is applied to any substance whose admixture with an injected immunogen increases the response.

The most effective adjuvants are the water-in-oil emulsions developed by Freund, particularly those in which living or dead mycobacteria are suspended (**complete Freund's adjuvant**). After a single subcutaneous or intramuscular injection (e.g., 0.5 ml in a rabbit) droplets of emulsion metastasize widely from the site of injection; Ab formation, detected as early as 4 or 5 days later, may continue for 8 or 9 months or longer (Fig. 19-15).

The intense, chronic inflammation around the deposits of emulsion precludes their use in man. However, emulsions without my-

FIG. 19-15. Influence of adjuvants: Schematic view of amounts of Ab produced by rabbits in response to one injection (**arrow**) of a soluble protein such as bovine γ-globulin, in dilute salt solution (**A**), adsorbed on precipitated alum (**B**), or incorporated in a water-in-oil emulsion containing mycobacteria (Freund's complete adjuvant, **C**).



cobacteria (**incomplete Freund's adjuvant**) are less irritating and have been used clinically; their enhancing effect is less than that of complete Freund's adjuvant. The adjuvant activity of the mycobacteria is due largely to a complex glycolipid, whose activity has been duplicated by a small, synthesized glycopeptide, **muramyl dipeptide**: N-acetyl-muramyl-L-alanyl-D-isoglutamine.

Most adjuvants act not only by increasing Ag persistence, but by somehow **increasing macrophage and T_h activities**. Some other adjuvants are killed *Bordetella pertussis*, the lipopolysaccharide (LPS) of gram-negative bacteria, and large polymeric anions (e.g., dextran sulfate). A few adjuvants (LPS, dextran sulfate) are **polyclonal B cell activators** and possibly act by promoting B cell proliferation.

Colchicine is unusual: at doses that evidently block proliferation of T_s cells preferentially it enhances Ab production, presumably by permitting unchecked T_h cell activity.

DOSE

Dose effects of Ag were discussed above, under Tolerance. It should be noted, however, that dose effects vary with conditions of immunization. For instance, with a typical protein injected in solution in a rabbit the smallest effective dose might be about 100μg, whereas injected in complete Freund's adjuvant it might be 1μg–10μg. Similarly, the threshold dose is usually much lower in previously primed than in immunologically "virgin" ("naive") animals. Moreover, a subthreshold dose can sometimes prime animals for a pronounced secondary response without actually eliciting Abs in a detectable primary response.

Aggregated versus Soluble Form. Protein Ags are more immunogenic when administered in aggregated than in soluble form. Thus chemically cross-linked protein molecules (e.g., by gluteraldehyde) and Ag-Ab complexes, prepared in slight Ag-excess (Fig. 16-7) are usually highly immunogenic. (When, however, the complexes are prepared in Ab-excess their immunogenicity is greatly reduced, probably in part because the antigenic determinants are blocked; see Suppression by Ab as Antiimmunogen, below.)

ANTIBODY TURNOVER AND DISTRIBUTION

The level of Ab in the serum reflects the balance between rates of synthesis and degradation. When the rates are equal, the serum Ab concentration is constant (**steady state**). The rate of synthesis depends upon the total number of Ab-producing cells, which varies enormously with conditions of immunization. By contrast, the rate of degradation (expressed as half-time, or $t_{\frac{1}{2}}$) is determined by the H chain class (Table 19-3): ***IgM and IgA are normally broken down much more rapidly than IgG molecules.***

However, infusion of a trace amount of ^{125}I-labeled IgG into individuals with widely varying IgG levels (from agammaglobulinemia or multiple myeloma) revealed an inverse relation between the half-time for IgG degradation and the total concentration of this Ig class: at high and low levels of IgG the $t_{\frac{1}{2}}$ was about 11 and 70 days, respectively, as compared with 23 days at normal levels. Injected Fab fragments and light chains disappear rapidly ($t_{\frac{1}{2}}$<1 day), but the Fc fragment has the same half-life as intact IgG. Analogy with some other serum proteins suggests that shortening of oligosaccharide branches, by random removal of terminal sialic acid or other residues by blood glycosidases, makes Ig molecules susceptible to uptake and degradation in liver macrophages (Kupffer cells).

TABLE 19-3. Some Metabolic Properties of Human Immunoglobulins

Properties	IgG*	IgA	IgM	IgD	IgE
Serum concentration (mg/ml) (average, normal individuals)	12.1	2.5	0.93	0.023	0.0003
Half-life (days)†	23	5.8	5.1	2.8	2.5
Rate of synthesis (mg/kg body weight per day)	33	24	6.7	0.4	0.016
Catabolic rate (% of intravascular pool broken down per day)	6.7	25	18	37	89

*IgG-1, IgG-2, and IgG-4 have the same half-life (~ 23 days), but that of IgG-3 is shorter ($t_{\frac{1}{2}}$ = 8–9 days), perhaps because the unusually large hinge region of γ3 chains (Fig. 17-15) increases susceptibility to proteolysis. The half-life of IgG in some other species is (in days): rabbit (6), rat (7), guinea pig (7–9).

†Half-life ($t_{\frac{1}{2}}$) is the time required for the concentration (at any particular moment) to drop to ½ the value; it is related to the first-order rate constant for degradation, k (in days^{-1}):

$$t_{\frac{1}{2}} \text{ (in days)} = 0.693 \; k.$$

(Based on Waldmann TA et al: In Immunoglobulins. Merler E. (ed): Washington, D.C., National Academy of Sciences, 1970)

The actual serum concentration of Ig also depends upon the volume in which the molecules are distributed. The total mass of IgG is about the same in blood and in extravascular fluids and about 25% exchanges between the two compartments each day.

CROSS-STIMULATION

A secondary response can sometimes be elicited with an immunogen that is not quite identical to the primary Ag. Surprisingly, most of the Abs made will then react more strongly with the first than with the second immunogen. This phenomenon, called **original antigenic sin**, was initially recognized in epidemiologic studies with cross-reacting strains of influenza virus.

For example, in rabbits that had been primed with Dnp-proteins, the secondary response evoked with 2,4,6-trinitrophenyl (Tnp)-proteins months later consisted primarily of Abs with the properties of anti-Dnp, rather than of anti-Tnp, molecules (Table 19-4). The effect is probably due to specific cross-stimulation, by Tnp-proteins, of an enlarged population of anti-Dnp memory B cells remaining after the primary response to the Dnp immunogen.

Once a clone has been triggered by one Ag, X, it can evidently be restimulated by a crossreacting Ag, X′, that itself is unable to stimulate a primary response of that clone. This principle has been exploited in **serologic archeology**, e.g., in testing human sera during an influenza epidemic with diverse strains of the virus. A given patient's serum tends to react less strongly with the strain

TABLE 19-4. Cross-Stimulation (Original Antigenic Sin) Illustrated in the Secondary Response to 2,4-dinitrophenyl (Dnp)-protein and to 2,4,6-trinitrophenyl (Tnp)-protein

Rabbit no.	Primary stimulus	Secondary stimulus	Affinity of antibodies formed 7-8 days after secondary stimulus: For 2,4-dinitrotoluene (DNT) (liters/mole × 10^{-7})	For 2,4,6-trinitrotoluene (TNT) (liters/mole × 10^{-7})	Ratio of affinities DNT/TNT
1	Dnp-BγG	Tnp-BγG	25.	0.49	50.
2	Dnp-BγG	Tnp-BγG	17.	0.85	20.
3	Dnp-BγG	Tnp-BγG	34.	0.84	40.
4	Tnp-BγG	Dnp-BγG	0.20	0.30	0.7
5	Tnp-BγG	Dnp-BγG	0.62	1.0	0.6

The primary stimulus was 1 mg Dnp-bovine γ-globulin (Dnp-BγG) or 1 mg Tnp-BγG; 8 months later animals with no detectable serum Ab were reinjected with the immunogen shown, and they produced Abs in abundance. In control rabbits given Dnp-BγG in both injections the ratio of affinities for DNT/TNT ranged from 2 to 100 (i.e., they formed **anti-Dnp** Abs). In other controls given Tnp-BγG in both injections this ratio ranged from 0.2 to 0.9 (i.e., they made **anti-Tnp** molecules). Most of the Abs produced within 1 week of the secondary stimulus had binding properties that correspond to the primary rather than the secondary immunogen.

(Based on Eisen HN et al: Isr J Med Sci 5:338, 1969)

causing his current illness than with the strain that caused his first attack of influenza in some previous epidemic. From the study of sera from very elderly patients it has thus been possible to identify strains that probably caused major epidemics in the past, e.g., in 1918, long before the influenza virus was discovered.

FATE OF INJECTED ANTIGEN

Following intravenous injection of a soluble Ag the decline in its concentration in serum exhibits three sharply distinguishable phases: 1) a brief **equilibration phase** due to rapid diffusion into the extravascular space, 2) slow **metabolic decay** during which the Ag is degraded, 3) rapid **immune elimination**, which identifies the onset of Ab formation; during this phase the Ag exists largely as soluble Ab–Ag complexes, which are taken up and degraded by macrophages. Free Ab appears at the end of the immune elimination stage.

Extensively phagocytized particulate Ags, such as bacteria and red cells, do not diffuse into extravascular spaces, and hence do not exhibit the initial equilibration phase of rapid decrease in serum concentration after intravenous injection. Trace antigenic fragments can persist in lymphoid tissues long after the Ag is no longer detectable in blood.

Do Ag-recognizing lymphocytes react with native proteins or with denatured proteins? Abs elicited by globular proteins react with native, not denatured, protein; hence B cells, which have Ab on the cell surface probably recognize native proteins, not protein that is denatured or fragmented by macrophages. However, some of the T cells that are elicited by globular proteins seem not to distinguish native and drastically denatured forms of the protein, suggesting that perhaps it is the primary amino acid sequence of the protein, bound and denatured and perhaps fragmented on macrophages, that is recognized by T cells. This unusual behavior of T cells is difficult to reconcile with other evidence that T cells and Abs (i.e., B cells) have similar idiotypes in their Ag-recognition sites (see Antigen-Binding Receptors on T Cells, Ch. 18) when they react with the same Ag, implying that T and B cells have similar Ag-binding activity and specificity.

A later chapter describes the extensive alterations undergone in vivo by some small molecules as they combine with tissue proteins to form immunogenic conjugates (e.g., penicillin, Ch. 22).

Antigenic Competition. The response to an Ag may be diminished if an unrelated Ag is injected at the same time or shortly before. For instance, rabbits injected with a foreign serum (say from the horse) produce Abs to serum globulin but not to serum albumin, although serum albumin alone elicits anti-albumin Abs. Similarly, poly-L-alanyl-protein readily elicits Abs to the L-polypeptide, but on coimmunization with poly-D-alanyl-protein only the latter elicits Abs to its polypeptide. This **intermolecular antigenic competition** is important in practical immunization programs (see Vaccination against Microbial Antigens, below), which often involve giving several different Ags in one vaccine. Adjusting the amounts of the several components ("balancing") can overcome the competitive effect.

Different determinants on the same molecule can also compete (**intramolecular competition**): thus a protein with both D- and L-polyalanyl peptides substituted on the same molecule evokes synthesis only of Abs to the D-polyalanyl groups. Presumably, the available cell surface receptors for the two determinants differ in affinity, and one determinant becomes dominant because the corresponding cells bind the limited supply of Ag. The mechanism for intermolecular competition is more obscure: it evidently involves a suppressive effect of T cells on B cells. Thus *the phenomenon is not observed in thymus-deprived mice unless they are given thymus cells.* However, the suppression is nonspecific in the sense that T_s cells elicited by Ag X can block anti-Y B cells.

INTERFERENCE WITH ANTIBODY FORMATION

SUPPRESSION BY ANTIBODY AS ANTIIMMUNOGEN

It was noted earlier (Aggregated versus Soluble Form of Ag) that an Ag is sometimes more immunogenic when administered as an

Ab–Ag complex, in slight Ag excess, than as the Ag alone. Usually, however, the addition of Abs to the immunogen is likely to block the induction of Ab synthesis, particularly when the added Ab is in relative excess, largely because the resulting Ab–Ag complexes are then degraded rapidly (see Fate of Injected Antigen, on page 438).

Inhibition by excess Ab is important clinically. For instance, severe hemolytic disease of the newborn, due to maternal Abs against fetal Rh^+ red cells (RBCs, see Rh Antigens, Ch. 23), has been greatly reduced in incidence by routine injections of anti-Rh Abs into Rh^- mothers at the time they deliver Rh^+ babies: the baby's RBCs, entering the maternal circulation in profusion as the placenta separates, are eliminated by the anti-Rh Abs. This prevents the mother from becoming immunized and reduces the risk of an anamnestic anti-Rh response during a subsequent pregnancy with another Rh^+ baby. Another example arises from placental transfer of maternal Abs to measles, polio, etc.: these can block the induction of Ab synthesis by the corresponding viral Ags in the young infant. Hence active immunization of the newborn is postponed until 6–9 months of age, by which time all maternal Abs have been eliminated (see Fig. 19-23).

SUPPRESSION BY ANTIBODY AS ANTIRECEPTOR

Abs to Ag-binding receptors on B or T cells can block the production of Igs of certain classes (isotypes) or allotypes or idiotypes, depending upon the specificity of the anti-receptor Abs and the conditions under which they are administered. The mechanisms are not well understood, but immunologic intervention of this type is a potentially powerful tool for manipulating immune responses.

Isotype Suppression. Injecting newborn mice with antiserum to μ chains leads to the absence of serum IgM, IgG, and IgA in the growing animal. Similar injections of anti-α or anti-γ antiserum block only the production of Igs with the corresponding heavy chains (IgA and IgG, respectively). The effects persist for several weeks, and recovery ensues gradually. Similar suppression cannot be established in adults, probably because their high levels of serum Igs neutralize the injected Abs.

Isotype suppression can also be shown in primary spleen cell cultures, in which the addition of both sheep red blood cells (SRBCs) and certain anti-Igs block the production of various classes of Abs to SRBCs: *antisera to μ chains inhibit the formation of Abs of all classes*, while antisera to γ1 or to γ2 chains block only the formation of those anti-SRBCs of the corresponding class.

These findings support the idea that *B cells with IgM on their surface are precursors of cells that secrete Igs of various classes* (see IgM–IgG switch, above, and B Cell Development, below).

Allotype Suppression. The first discovered case of suppression by anti-Ig was found by Dray among hybrid offspring of crosses between rabbits with different Ig allotypes (see Ch. 17, rabbit *a* and *b* allotypes of heavy chains and of κ light chains, respectively). If the newborn receives antiserum to the paternal allotype, it produces hardly any Ig of that allotype for many months; however, a compensating overproduction of the maternal allotype maintains the total Ig at a normal level (Fig. 19-16). Antiserum to the mother's allotype is not effective in the newborn, whose high levels of maternal Ig, acquired transplacentally or by suckling, neutralize the injected Ab. (Antiserum to the father's allotype is suppressive only if it is given before a neutralizing level of this allotype has been actively produced, i.e., before the third week after birth in rabbits.)

Newborn rabbits that produce no κ-containing Igs at all can also be elicited by Abs to κ-chain allotypes: e.g., by injecting into a foster mother who has one kind of κ allotype, and who carries implanted fetuses with a different κ allotype, Abs to the fetus's allotype. The total serum Ig level in the resulting κ-suppressed newborn is normal, because all of its L chains are λ type (which is ordinarily present in only about 10% of rabbit Ig molecules).

Like all Ig molecules that circulate in vivo, the suppressing Abs

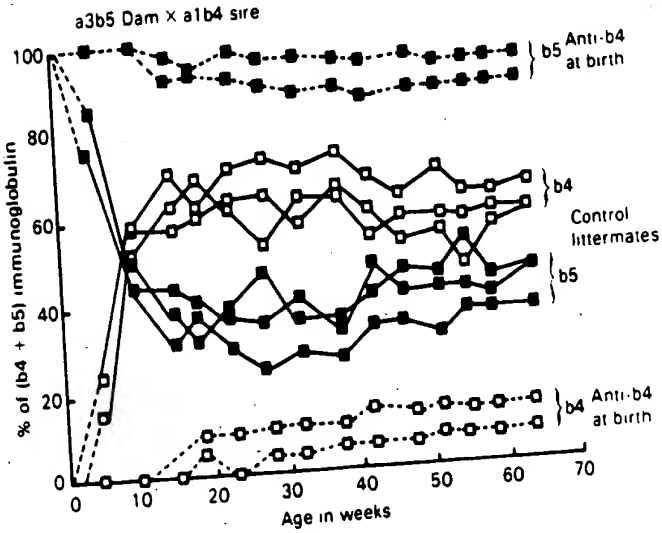


FIG. 19-16. Allotype suppression in rabbits. The parents were homozygous for different light-chain allotypes (father, b4/b4; mother, b5/b5). At birth half the offspring (b4/b5) were given 2.2 mg anti-b4 Abs (---); control littermates did not receive Abs (———). For over 1 year, the target allotype (b4) was virtually lacking in the treated animals' Igs while the other allotype (b5) was overproduced; the level of total Ig in serum was normal as were the heavy-chain allotypes. The changes in control rabbits at 5–10 weeks are due to onset of synthesis of the paternal allotype (b4□) and loss of passively acquired maternal Igs (b5,■). Relative concentrations of b4 and b5 in controls are not unusual for products of codominant alleles. Levels of individual allotypes are expressed as percent of the sum of both allotypes (b4 + b5). (Mage RG: Cold Spring Harbor Symp Quant Biol 32:203, 1967)

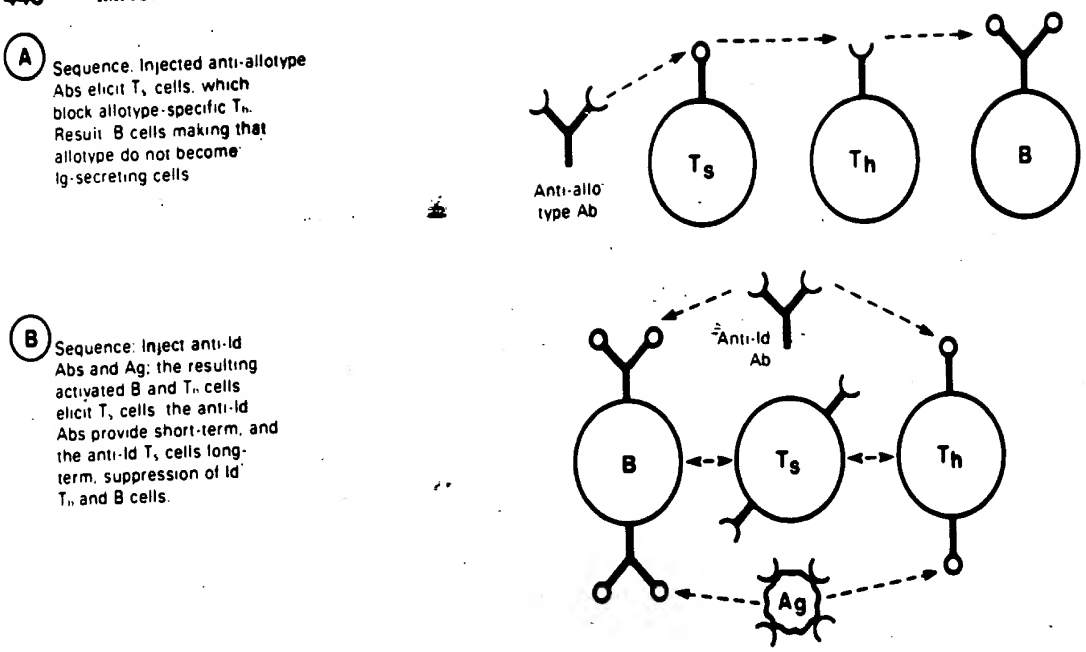


FIG. 19-17. Hypothetical schemes for Ab-triggered suppression of the production (by inbred mice) of particular Igs. **A.** Allotype suppression. Injected antiallotype Abs elicit T_s cells, which block allotype-specific T_h. As a result, B cells making that allotype do not become Ig-secreting cells. **B.** Idiotype suppression. Anti-Id Abs and Ag are injected; the resulting activated B and T_h cells elicit T_s cells: the anti-Id Abs provide short-term, and the anti-Id T_s cells long-term, suppression of Id$^+$ T_h and B cells. In A the antiallotype Abs were from allogenic mice. In B the antiidiotype Abs were from rabbits. (A, based on Herzenberg et al: Cold Spring Harbor Symp Quant Biol 41:33, 1976; B, based on the Ju S-T et al: Cold Spring Harbor Symp Quant Biol 41:699, 1976)

T_s cells seem to be a complex family of cells. In some responses, Ag stimulates T_s-1 cells to release a soluble factor, T_s-1F, that binds Ag and, from its serologic properties, appears to have polypeptide sequences encoded both by Ig V genes (V_H) and by genes of the major histocompatibility complex (IJ of the mouse H-2 complex). T_s-1F stimulates T_s-2 cells to produce another factor, T_s-2F, which causes still other T_s cells, T_s-3, to act with broad specificity on diverse effector lymphocytes.

are broken down in a few weeks (Table 19-3). However, the suppressive effect persists for many months, because the Abs somehow trigger the development of specific T_s cells. The mechanisms are not known but one possibility is considered in Fig. 19-17A.

Idiotype Suppression. Abs to an idiotype (anti-Id) can suppress production of Igs with that idiotype. Thus, rabbit Abs to the shared idiotype that is present on a high proportion of the anti-arsenate (anti-ARS) Abs made by A-strain mice can block the production of these anti-ARS Abs and not of other anti-ARS Abs made by the same mice. The suppression is induced by injecting mice with the anti-Id Abs (from rabbits) and then immunizing them with an ARS-protein conjugate: anti-ARS Abs are produced, but they all *lack* the shared idiotype. The suppressed mice develop T_s cells that bind Id$^+$ anti-ARS Ab molecules but not Id$^-$ anti-ARS molecules. The suppressive activities of these T_s cells are, perhaps, exerted directly on B cells with surface Id$^+$ anti-ARS, or on T helpers with the same Id on their surface receptors (Fig. 19-17B). T_s and B cells that share the same Id are probably specific for the same determinant (in this instance the benzenearsonate group).

NONSPECIFIC IMMUNOSUPPRESSION

Whole-body x- (or γ-) irradiation and a variety of cytotoxic drugs (Fig. 19-18) can prevent the initiation of Ab formation by any Ag. But once Ab synthesis is under way they cannot interrupt it, unless given in doses that are severely cytotoxic for cells in general. *All these agents are much more effective in blocking the primary than the secondary response:* either memory cells are more resistant than virgin Ag-sensitive cells, or the increased number of cells that can respond increases the probablity that some will initiate Ab formation before they can be blocked. Some immunosuppressants act primarily as inhibitors of cell division (**antiproliferative**, e.g., the antimetabolites); others act primarily by destroying lymphocytes (**lympholytic**, e.g., 11-oxycorticosteroids); and others are both lympholytic and antiproliferative (x-rays, radiomimetic alkylating agents).

Most of the **antimetabolites** (purine, pyrimidine, and folate analogs; Chs. 7 and 11) were developed as byproducts of cancer chemotherapy screening programs. Their principal action is to **block cell proliferation**, and they are especially toxic for rapidly dividing cells—in tumors, bone marrow, and intestinal and skin epithelium, as well as lymphocytes that proliferate in response to stimulation by Ag. Accordingly, the margin of clinical safety between therapeutic and toxic doses is small, but for some of these

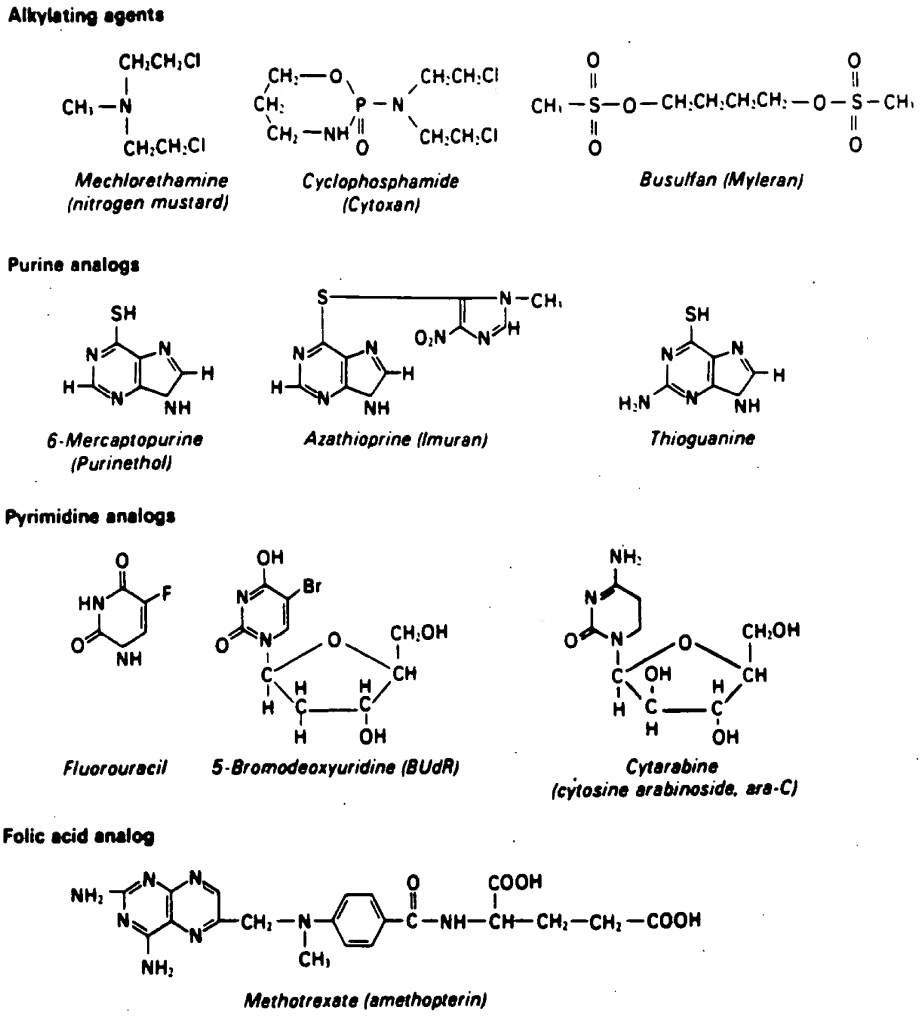


FIG. 19-18. Some immunosuppressive drugs. Azathioprine (Imuran) is converted in vivo to 6-mercaptopurine.

drugs it is great enough for routine clinical use (e.g., azathioprine and cyclophosphamide).

The frequency of successful kidney transplants in man is now largely due to skillful use of immunosuppressive agents: currently favored are combinations of azathioprine (an antimetabolite), prednisone (a corticosteroid), and antilymphocytic serum (ALS) or its IgG fraction (ALG), which selectively destroys circulating T cells (see Chs. 22 and 23).

Lympholytic agents (x-rays, radiomimetic alkylating drugs, corticosteroids) cause prompt and massive destruction of lymphocytes. The chromosomal damage caused by x-rays and alkylating agents also impairs the capacity of surviving lymphocytes to undergo mitosis, blocking their normal response to subsequent antigenic stimulation.

In the following comparison of various agents it is useful to contrast their activity in the three phases of the Ab response: 1) the preinductive phase, before immunogen is administered; 2) the inductive phase, between immunogen administration and rise in titer of the corresponding serum Ab; and 3) the productive phase, when Ab is being synthesized vigorously. *The lympholytic agents are effective immunosuppressors when given in the preinductive phase, and the antiproliferative agents are most effective in the inductive phase.* All known agents appear to be ineffective in the productive phase, unless given in highly toxic doses. Though this division into phases is convenient, it is an oversimplification, for lymphoid cells do not respond synchronously: the immunogen can remain active for months, and cells in all three phases may be present simultaneously.

X-rays. Whole-body irradiation with sublethal doses of x-rays (400–500 r) can suppress the response to most immunogens for many weeks, but usually not permanently. Suppression is greatest when the irradiation is given 24–48 h before the Ag, because mas-

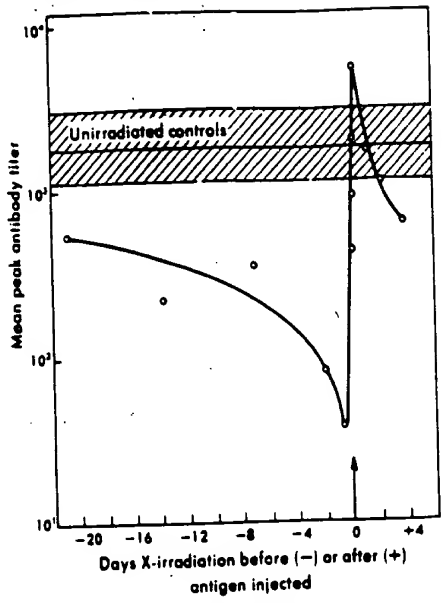


FIG. 19-19. Effect of 500-r x-irradiation of rabbits at various times before and after immunization **(arrow)** with sheep red cells. Ab titers were measured about two weeks after the injection at time zero **(arrow).** (Based on Taliaferro WH, Taliaferro LG: J Infec Dis 95:134, 1954)

sive disintegration of lymphocytes occurs promptly after whole-body irradiation. After a large dose (e.g., 500 r) active proliferation of lymphoid cells is resumed after about 3–4 weeks, and lymph nodes appear normal shortly thereafter. However, damage to chromosomes (the main targets of x-ray) can be "stored": i.e., it may not be expressed until the cells attempt to divide, perhaps months or years later. In the meantime some *nondividing cells (e.g., T helper cells) can apparently function normally.*

When an immunogen is given after massive irradiation it may be eliminated before the capacity to initiate Ab formation is restored. However, if the immunogen is given just **before** irradiation the stimulated cells can apparently continue their differentiation and eventually forms Abs, while already differentiated cells continue to synthesize Abs. The responding cells may even yield more Ab in animals irradiated 1-2 days after immunization (Fig. 19-19), perhaps because T_s cells are more readily inactivated than T_h or B cells by small doses of x-rays.

The secondary response is relatively resistant to irradiation: the appearance of Abs may be delayed, but peak titers are usually **normal.** As in the primary response, Ab formation may be increased by irradiation given shortly after the booster injection.

Alkylating Agents. Compounds such as the nitrogen mustards (Fig. 19-18) block cell division by cross linking strands of DNA. Often called **radiomimetic drugs**, their biologic effects (such as massive destruction of lymphocytes) resemble those of x-irradiation. However, recovery is more rapid than after x-rays, and these drugs are therefore usually given at frequent intervals to bring about sustained immunosuppression.*

Corticosteroids. Large doses of 11-oxycorticosteroids cause extensive destruction of small lymphocytes. However, the surviving small T cells appear to be unusually active in some reactions, e.g., graft-versus-host (Ch. 23). Nonetheless, if the steroids are given just before the Ag they inhibit Ab formation in some species (rats, mice, rabbits); but at the doses used clinically a significant suppression has not been observed in man. In therapeutic doses these drugs inhibit inflammation, whether due to allergic reactions (Chs. 21 and 22) or to nonspecific irritants such as turpentine. Accordingly, they are widely used clinically to suppress allergic inflammation, especially if it is persistent (as in some types of bronchial asthma).

Antimetabolites. In contrast to x-rays, which are most inhibitory when given just before the immunogen, the antiproliferative metabolite analogs, such as 6-mercaptopurine, usually suppress Ab formation best when their administration is begun 2 days afterward, when the Ag-induced proliferation of lymphocytes is particularly active (see Selectivity, below). The difference is readily understood: x-rays (and alkylating agents) can damage DNA whether or not it is replicating, while the *immunosuppressive antimetabolites damage only cells with replicating DNA.*

If **dactinomycin** is added together with the immunogen the formation of Ab is substantially inhibited, because the synthesis of new mRNA is required for initiation of Ab synthesis. However, once Ab formation has started it can persist for many days in cell culture in the presence of this drug, suggesting that the corresponding mRNA is relatively stable.

Selectivity. Though immunosuppressants generally affect immune responses as a whole, rather than particular manifestations, some selective suppression has been observed. For instance, x-rays, corticosteroids, and antiproliferative drugs suppress IgG production more than IgM, suggesting that fewer cell divisions may be needed to initiate production of Abs of the IgM class. As will be noted later (Ch. 22), antisera to lymphocytes (ALS, ALG) seem to block cell-mediated more than humoral immunity.

Selectivity has also been brought out by combining the administration of Ag and antiproliferative immunosuppressant therapy. The clones whose proliferation has been stimulated are selectively eliminated by the drugs, as in the selective destruction of growing bacterial cells by penicillin.

* At low doses these agents (e.g., cyclophosphamide) are particularly damaging to T_s cells and they can, therefore, enhance immune responses.

Complications of Immunosuppression. Prolonged immunosuppression is dangerous, not only are the agents highly toxic for various cells, but they can activate latent infections and greatly increase susceptibility to serious infection with prevalent opportunistic fungi, bacteria, and viruses that ordinarily have little pathogenicity (e.g., *Candida, Nocardia,* cytomegalovirus, herpesvirus). In addition, chronically immunosuppressed individuals, like those with congenital immunodeficiencies (see Immunodeficiency Diseases, below), have an increased incidence of certain cancers. Suppression of cell-mediated immunity rather than of Ab formation is probably responsible, because effective immune responses to most tumors seem to be cell-mediated (Ch. 23).

B CELL DEVELOPMENT

PRE-B TO MATURE B CELLS

B lymphocytes, like other blood cells, arise from pluripotential hematopoietic stem cells. In embryogenesis, the first recognizable cells of the B lineage make μ chains but not L chains. More differentiated cells, called **pre-B cells,** are detected in the fetal liver about half way through gestation. They appear as large and small lymphocytes with cytoplasmic IgM but no surface Ig molecules.* Without further maturation in vivo or in vitro they are not responsive to mitogens or to Ags. Similar cells are seen in small numbers in adult bone marrow, indicating that B cells continue to arise from stem cells throughout life.

Pre-B cells give rise to **immature B cells,** which have surface IgM (sIgM$^+$) but no cytoplasmic Ig (Fig. 19-20). They lack several of the surface molecules that are characteristic of mature B cells (Ia, FcR [receptors for the Fc domains of aggregated Igs; Ch. 17], and C3R [receptors for the C3b fragment of complement; Ch. 20]); and, as noted before, they are highly susceptible to the removal of surface Ig by multivalent Ags or by Abs to surface Ig molecules (see Modulation, under Clonal Deletion, above).

Mature B cells arise from the immature ones. Besides sIgM, the mature cells usually have one or two other surface Ig isotypes: most have sIgM + sIgD, and some have sIgM + sIgG or sIgM + sIgA.

From the percentage of cells that bind fluorescent Abs to various isotypes it appears, but has not been demonstrated directly, that some cells with sIgM + sIgG, or with sIgM + sIgA, also have sIgD. Only infrequent mature B cells have a single surface Ig isotype (usually IgM, sometimes IgD or IgA). Mature B cells also have surface Ia molecules and receptors for Igs (FcR) and for a complement fragment (C3R). As noted before (see Tolerance, above) these cells, unlike immature B cells, are relatively resistant to removal of sIg molecules by multivalent Ags and by anti-Ig Abs, and they are not easily made tolerant.

As noted above, the **terminal differentiation steps** in which mature B cells are stimulated by T-dependent Ag to develop into **plasma cells** (which secrete IgG or IgA), and into **memory cells,** are greatly influenced by T_h cells and macrophages. In contrast, the maturation step in which immature B cells begin to express surface Ig isotypes other than IgM seems not to depend upon either T cells or Ags, for it occurs normally in athymic mice raised under pathogen-free conditions.

In human fetuses lymphocytes with IgM or IgG on the cell surface are detected by immunofluorescence by the 10th week of gestation, and cells with surface IgA are detected slightly later. By the 15th week the proportion of fetal spleen and blood cells with each Ig class is essentially the same as in the normal adult. However, secretion of Igs by fetal cells occurs much later, perhaps because T helpers are absent or because restricted diversity of Ag-sensitive cells and of foreign Ags in the sheltered fetus reduces the chances for Ag-triggering of the fetus's lymphocytes. IgM and lesser amounts of IgG normally begin to be synthesized and secreted by fetal spleen cells in about the 20th week, and production of IgA seems to start only some weeks after birth. However, with severe fetal infection, as in congenital syphilis, Abs (of the IgM class) are formed vigorously, and plasma cells are abundant in the infected 6-month fetus (Fig. 19-21).

The central role of IgM-bearing cells in the maturation sequence is also evident from inhibition experiments. Abs to μ chains, administered to newborn mice, block the development of all Ig-producing cells and result in agammaglobulinemic mice, whereas Abs to γ or to α chains block the production of just IgG or IgA molecules, respectively.

Taken together, all these results suggest that the order in which Ig H chains are produced in embryogenesis repeats the apparent evolutionary sequence ("**ontogeny recapitulates phylogeny**"): *IgM, then IgG and IgA* (see Ch. 17, Fig. 17-40).

Acquisition of Specificity. The initial synthesis of Ig marks the transition from stem cells to pre-B cells. The transition probably requires gene rearrangements that

* Immunofluorescence is used to detect Igs in or on cells. Extracellular Abs (labeled with fluorescein or rhodamine) can penetrate into dead cells, but not into living ones. Accordingly, cytoplasmic Ig is revealed by staining "fixed" (i.e., killed) cells with fluorescent Abs, and surface Ig is revealed by staining living cells (see Fig. 18-10, Ch. 18).